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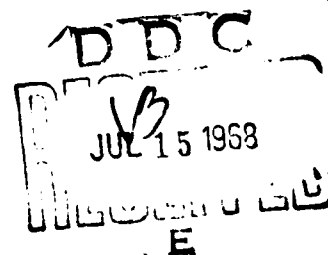
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## INVESTIGATIONS ON DEFOLIATION AND RELATED PHENOMENA

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With 1 table and 14 text illustrations.

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### I. Main Results of Past Anatomical Investigations on the Absciss Layer

A short time after Mohl's treatise on the absciss layer (Bot. Zeit. (Botanical Journal), 1860, pp 1-7 and 9-17) a second work by the same author was published on the separation of juicy plant organs (Bot. Zeit., 1860, pp 273-277); in this second work the author proved that all leaves belonging to the blossom region, as well as the axis (young branch tips, Phyllocladia, blossom stalks) are separated in the same way as deciduous leaves; it was therefore obvious to generalize this phenomenon and to trace each and every plant separation process to the formation of an absciss layer with cells falling apart all around; today we still customarily think only of this process when we talk about the absciss layer. Mohl's investigations relate almost exclusively to the dicotyledon plants. Bretfeld (Pringsheim's Jahrb. f. wiss. Bot. (Pringsheim's Yearbook of Scientific Botany), Vol XII, 1879-1881) showed for a number of groups of monocotyledons that defoliation [the fall of leaves] is caused through growth processes in the epidermis and in the basal tissue; more specifically, this occurs in a layer which exists from the very beginning of leaf development and which is particularly differentiated.

(Mohl (1) says that it is characteristic for the absciss layer that it develops only shortly before defoliation along a place which in no way whatever differs, until then, from the remaining basic [basal] tissues; but in exceptional cases (in the case of *Sedum maximum* and in a fern *Woodsia ilvensis*) there is a kind of pre-formed absciss layer in the form of a small-cell zone which is between the tissue of the blossom stalk and the axis. On the basis of the process described by Molisch, we want to use the term "absciss zones", using the expression coined by Hohnel (1) in referring to formations of this kind.)

In tree-like forms (*Aletris*, *Yucca*, *Dracaena*), the cell walls in the absciss layer become thicker while in the case of the *Orchidaceae* and *Aroideae*, at the time of defoliation, it is first of all the cells of the epidermis, facing those of the Mesophyll whose growth appears to be inhibited; then the zone of Sklerenchyma cells, which separates the part to be cast off from the part that remains behind, is subdivided into two layers

by means of newly-formed cells; in all cases leaf separation occurs when the delicate-wall and the thick-wall cells move away from each other, presumably due to uneven surface growth of the elements that border on each other; the situation is similar in many conifers (Molisch) (Untersuchungen über den Laubfall (Investigations on Defoliation), 1886, pp 32 ff.; *Taxus* does not have an absciss zone and an absciss layer has not yet been observed here either).

According to Mohl, the parenchyma cells move apart and break up as a result of all-round rounding-off and the vascular bundles are torn apart by outside forces; on the other hand, Tison (Recherches sur la Chute des Feuilles (Research on Leaf Fall), 1900) found in a large number of dicotyledons that the process of separation consists primarily in that the middle lamella and the thickening layers are dissolved between two cell layers of the absciss layer; at the same time we often observe a stretching of the cells involved in the longitudinal direction. This causes the vascular bundles to be torn. But Wiesner (Untersuchungen über die herbstliche Entlaubung (Investigations on Autumn Defoliation) discovered as early as 1871 that the vascular bundle on the level of the absciss layer is poor in mechanical elements and that the vessels in this particular place have a smaller cross section which makes tearing easier at this point.

## II. On the Mechanism of Leaf Separation /Defoliation/

(The data in brackets [...] refer to the experiments mentioned in Section X of this article.)

If, immediately after defoliation, we look at the two exposed surfaces under the microscope, we very often get a rather strange picture; both of these surfaces are covered with spherical isolated separation cells. This happens for instance in the case of the following: *Ampelopsis hederacea*, *Ligustrum vulgare*, *Evonymus europaea*, and among the evergreens: *Goldfussia isophylla*, *Aucuba japonica*, *Eugenia Ugni*, *Elaeagnus flexa*, *Camellia japonica*.

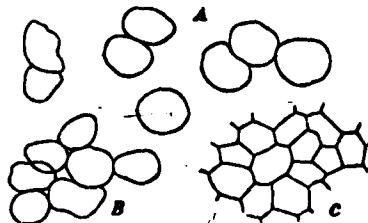


Figure 1. Enlarged 104 X. *Philodendron pertusum*. A, B -- cells of the absciss layer in various stages of isolation. C--a piece of normal basal tissue (directly under the absciss layer), seen from the surface (looking parallel to the defoliation star).

These cells, which have become spherical as a result of the turgor make it probable that the process of separation is primarily a mechanical one which is completely secondary in nature compared to the maceration of the middle lamella. (I believe that I may conclude this from the fact that when the maceration is very clear and extends over many cell layers, the cells thus released often are not spherical but rather assume a wide variety of shapes.) Mohl observed only this mechanism. Suitable preparations indicate how the surface, along which neighboring cells touch, shrinks as the rounding-off process progresses (Figure 1) -- a circumstance which so considerably reduces the strength of the leaf stem that a very small and later on quite insignificant external force can cause the leaf to fall off. Very often the absciss layer is on the outside recognizable macroscopically as a fine furrow surrounding the leaf stem; the ring-shaped depression is created by the fact that the cell multiplication in the absciss layer leads to the tearing of the epidermis; when the cells begin to become rounded off, the furrow widens into a gradually deeper-cutting split or cleft (Figure 2, a a' b) from which the isolated cells well out.

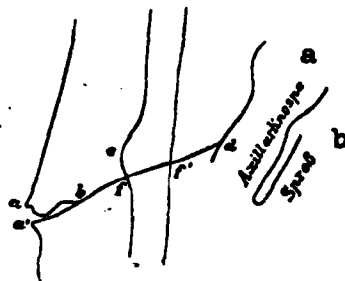


Figure 2A. Enlarged 30 X (see below for explanation). Absciss layer of *Goldfussia isophylla*.

Legend: (a)-- axillary bud; (b) -- sprout.

The process which Tison mentioned earlier is quite a bit different from this one; in this process, the participating cells along the surface to be released or cleared in the end are delimited only by a very thin membrane; this happens between two cell layers of the permanent tissue due to the dissolution of the middle lamella and the thickening layers. The process of cellulose dissolution ends the connection between the organ to be jettisoned and the sprout; the thin-wall cells which often become rather elongated (they are most heavily elongated, according to Tison's illustrations, in the case of *Amorpha fruticosa*), after the fall of the leaf, cover the two released or exposed surfaces along which they adhere with those portions of their membrane which are not thinned out. The difference between this one and the first-mentioned mechanism lies in the fact that the absciss layer is formed without any all-round cell isolation and without a follow-up meristema. (According to Mohl, the absciss layer usually develops out of a follow-up meristema; in some plants, as in *Brugmansia candida*, he was unable to establish any cell subdivision; but, since he speaks of a

rounding-off of the cells, *Brugmansia* undoubtedly drops its leaves by means of the first mechanism. A rounding-off of the separation cells is possible only in thin-wall cells, a condition which is met primarily in the case of meristematic tissue. In the delicate-wall tissue of flower leaves (Mohl, II) the formation of the absciss layer consists in a chemical change in the cell content and -- without there having been a multiplication of the cells -- it also consists in the rounding-off of these cells under more or less considerable magnification or enlargement. By the way, the process connected with the fall of flower leaves is not always identical with our first mechanism. From the investigations by Kubart (Organ. Ablosung der Korollen (Organic Separation of Corolla), 1906) we can see that the maceration here is very often quite emphasized (especially in the case of *Imatophyllum*, *Fuchsia*, *Nicotiana*) and that the increase in the turgor tension assumes second place here. But in the separation of deciduous leaves likewise we run into the maceration mechanism, more specifically, in its purest form in connection with the freezing of the absciss layer because at that time the turgor tension is altogether impossible due to the fact that the protoplasm has been killed, as a result of which it becomes simultaneously passable for organic acids (Wiesner, VI, page 57). (For the role of organic acids during defoliation, see also Wiesner I, page 39 and Figure 4, as well as Wiesner, VI, page 55 f.).)

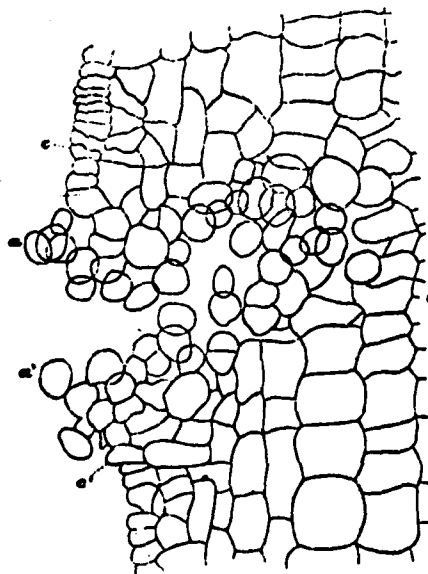


Figure 2B. Enlarged 200 X. Absciss layer of *Goldfussia isophylla*.

Legend: aa'd Absciss layer.  
ff' Absciss layer within vascular bundle, considerable enlargement, mainly recognizable along the place where the spiral band of the vessels is stretched.

- e Swelling of vascular bundle directly above absciss layer, probably due to multiplication of parenchymatic elements between the vessels (not constant).
- aa'b crack filled with round cells, progressing at b.
- a,a' round-cell masses, welling up.
- c,d' epidermis cells which were originally in contact with each other and which were separated from each other by the growth of the absciss layer.

I was able to observe a rather strange mechanism in some of the evergreen deciduous trees and I described this in a preliminary report ("On a Strange Anatomical Change in the Absciss Layer During the Defoliation of Leaves," *Ost. bot. Zeitschr.* (Austrian Botanical Journal), No 10, 1906, pp 380-385). The separation results when the follow-up meristema cells of the mostly acroscopic cell layer of the absciss layer (Figure 6), and under some circumstances also the cells of the next-lower cell layer (Figure 9), begin more or less to proliferate and become separated from each other through the shift of the membranes against each other (sliding growth; progressing separation can be seen, for instance, Figure 7), without there being any noticeable chemical action which might dissolve the middle lamella. Of course, I do not want to deny or doubt that, by way of a parallel support, there might also be some kind of chemical agent present which might loosen the connection between the young, thin-wall cells; but it does not take any profound cellulose dissolution or maceration of the otherwise quite thin membranes because the mechanical force of the shift is sufficient to separate the elements of the youthful tissue from each other. The most striking difference between this mechanism and the previously described first mechanism we note, however, right after the leaf has fallen, along the exposed defoliation wound: as a rule, this wound is covered with hose-shaped cells (Figure 6) which remain firmly connected on their basal end with the meristema from which they originated. The free end frequently is swollen up in the form of a knob. The exposed leaf stem surface reveals the unchanged parenchyma cells which break up with smooth walls; the membranes of the cells are very thin on the exposed side. This thinning, as we shall explain later, is not due to the dissolution of the thickening layers. The separation tissue remains completely at the sprout while, in the other mechanisms, the separation takes place in such a way that each of the two exposed surfaces has a portion of the absciss layer (Van Tieghem); of course, here again the major portion remains behind on the leaf cushion (Tison, page 267).

(The authors do not sufficiently differentiate the structure of the absciss layer and the process (mechanism) directly leading to the separation; only Wiesner, in a treatise (VI) goes into detail in the mechanics of defoliation. Mohl and Van Tieghem are familiar only with the rounding-off mechanism but Tison does not have anything at all to say about the rounding-off of the cells in his summary (page 264 ff) although he also investigated plants which undoubtedly involve the same mechanism; instead, he describes merely the dissolution mechanism which occurs only in some of the plants he investigated. Tison's subdivision of the absciss layers into

various types is primarily morphological and only very rarely mentions the separation process as such. We will come back later to the mechanism of the first type (Section IX) which is quite different. In a work by Van Tieghem and Guignard (see Bibliography) we can read about the defoliation of *Gymnocladus canadensis* in an absolutely humid moist space; in this connection there is a reference to a process which interpreted as a "resorption of a cell layer"; this process would appear to be identical with Tison's dissolution mechanism or it would at least be very closely related to it.)

In connection with another mechanism, we will discuss the anatomical conditions with the help of an example (*Nerium*) in Section V; this is a phenomenon which was for the first time investigated physiologically by Wiesner (Über Frostlaubfall, etc (On Frost Defoliation, etc), Type c); here the leaf stem, which has dried up to the absciss layer, due to the turgescence of the remaining stump, loses contact with the latter (see Section X, (VIII, 1)).

The previously mentioned properties of the absciss layer refer only to the basal tissue. The epidermis is torn into two cell layers along the boundary; this seems to be the case usually (Figure 2B and 3); but we also find cells torn, with the opening of the lumen (Figure 13,e). As far as the vascular bundles are concerned, I would like to add to what I said in the preceding section only that the tearing of the vascular bundles should be understood completely as an organic process and that this does not involve any outside force, not even gravity, as Van Tieghem assumes and as Wiesner (I, page 4) was able to observe occasionally; I want to point out that the cells of the absciss layer pushed their way between the mechanical elements and that individual vessels as a result were torn even before the fall of the leaf whereas cambium cells in the area of the absciss layer of the vascular bundle fell apart just like the parenchyma cells of the basal tissue.

(One experiment which I was always able to make with the same success showed the force of the proliferating separation cells alone is capable of tearing the vascular bundle. Small pieces of sprouts of *Evonymus japonica*, consisting of two half internodes and pertinent leaf tear, were planted with one of the cutting surfaces in a glass cup, lined with moist cotton, and were set up protected against concussion and air drafts. In order to eliminate the force of gravity as much as possible, I trimmed the blades on some leaves. After less than one week, all leaves had fallen off, the bladeless stems, however, remained in contact with the sprout on a very small piece of epidermis, opposite the axillary angle. Between the two exposed surfaces there was a deep gap. In the samples that were set up upside down, with the upper cutting surface against the cotton, the leaf stems had performed a movement upward, in other words, against the direction of the force of gravity, so that we can certainly cross off the force of gravity as one of the contributing factors in defoliation.)



Under small magnification we can often recognize that the differentiation which constitutes the absciss layer and which becomes recognizable with the naked eye along the longitudinal section as a straight, differently colored line, also runs through the vascular bundle (Figure 2A, ff'); under large magnification we can then, in favorable cases, see that the vessels are heavily stretched on the level of the absciss layer of the individual spirals of the thickening lasts and it is clear that, as the absciss layer continues to develop, the stretched spirals finally are torn. But in leaves which were separated from the leaf stem from the base on (citrus species, see page 1002) I was also able to see tracheal elements with smooth walls, breaking up and falling apart.

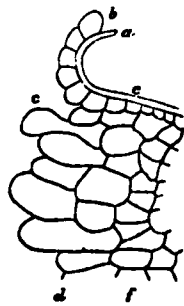


Figure 3. Enlarged 200 X. *Evonymus japonica*, upper edge of defoliation wound (material taken from III,6).

Legend: a -- cuticula  
b -- epidermis cells  
cd -- separation cells  
ef -- boundary of absciss layer against unchanged basal tissue

### III. Development of Absciss Layer Leading to the Hose-Cell Mechanism

It happens not infrequently in the vegetable kingdom that cells of a tissue layer begin to proliferate in response to some kind of stimulus and reach many times their normal diameter especially in the longitudinal direction. This is almost always a pathological phenomenon. This kind of volume increase can be found, after the injury to an organ, frequently in the cells directly under the wound surface; they form callus hypertrophies (Kuster, Pathologische Pflanzenanatomie (Pathological Plant Anatomy), pp 91 ff). By injuring leaves, Kuster was able to achieve a tremendous increase in the mesophyll cells; the cross section through the wound edge of a *Cattleya* leaf, which is shown in his work, is very similar with our sections through the fresh defoliation wound of plants which use the hose-

cell mechanism for defoliation. It was therefore obvious to assume that the hose-cells of the absciss layer are formations which develop as a result of an injury, of course, a physiological injury. In *Cinnamomum Reinwardti* I was indeed able to cut the leaf whenever the leaf had fallen off due to external factors, involving perhaps very little force, or as part of an experiment; here I was often able to find hose-cells. The absciss layer consisted simply of newly-formed, thin-wall cells which broke up with smooth walls. Fresh wounds on spontaneously dropping leaves, however were without exception covered with hose or tube cells. On the other hand, in *Cinnamomum Camphora*, the hose or tube cells were developed already at a time when the leaf was still relatively strongly attached but when it fell as a result of a minor impact or due to careless contact.

The entire development however could be followed most completely with the help of *Laurus nobilis* and this made it possible to remove any doubt that the tube cells are not just a wound reaction but that they have a certain specific function, that is to say, to lift the leaves from the sprout and thus to tear the vascular bundles.

The first change in the leaf joint of *Laurus nobilis*, which leads to the development of the absciss layer, is a change in the physical and chemical properties of a small membrane portion of the cells from which the meristema is to emerge; at the same time their lumen [inside diameter] appears to be filled by a somewhat denser content; the cell walls well up, become more transparent and stain less well when the preparations are stained. (Remnants of membranes thus altered can still be found in older stages; for instance, in Figure 4, where the meristema formation is already in progress, the membrane pieces fg and hi stained rather poorly; they looked rather fuzzy and appeared to grow narrower toward one side.) As we look at it three-dimensionally, the part of each cell which is affected by this change (for instance, acdf in Figure 4 -- here again we already have another change, that is to say, the growth of the membrane with the thinning portion), presumably looks like a ring-shaped body which cuts the cell into two parts which, in turn, after the subdivision of the nucleus, are separated from each other by a membrane. The rather swollen-looking wall portion of the mother cell becomes thinner and the cell begins to proliferate so that the two partitioned products, resulting from its two poles, move further apart.

The previously mentioned ring then develops into a long, thin-wall cylinder which falls apart into several cells as a result of cross-wall formation. In this stage we thus have a layer of thin-wall cells (see Figure 5 -- in *Laurus nobilis* the corresponding stage is developed in the case of sprouting defoliation but it is not developed in connection with sporadic defoliation (see Figure 6)) which can be noted with the naked eye as the transparent and very clearly visible line on the cross section. The membranes of the meristema, especially those running longitudinally, develop folds as a result of the fact that they grow in a limited space.

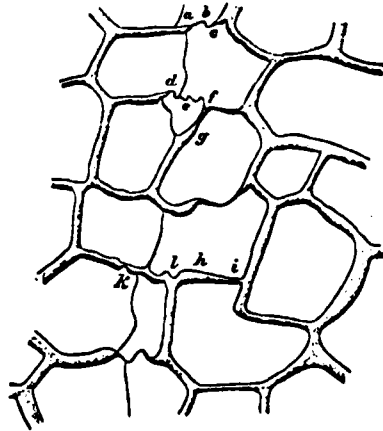


Figure 4. Enlarged 520 X. *Laurus nobilis*, first beginning of absciss layer (drift leaf fall). The membrane portions ac and df are in a stage of increased growth and, while becoming thinner, develop folds (b, e) because of the limited space available. The thin membrane sections at k and l are perhaps the places that happen to be suitable for this kind of growth. For fg and hi, see above.

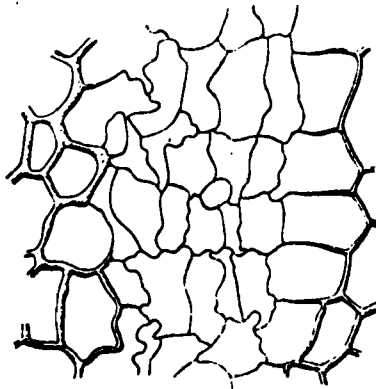


Figure 5. Enlarged 255 X. *Cinnamomum Reinwardti*, absciss layer (sporadic defoliation).

The tension which results from the cell multiplication finally causes the epidermis, which is not actively involved in this, to burst and the tear can be seen very clearly as a ring-shaped furrow which separates the leaf stem from the leaf cushion. The vascular bundle likewise undergoes a change in that the parenchymatic elements participate in the formation of the absciss layer and in that mechanical elements, in combination with the new formations emerging from the basal tissue, individually, partly after prior crushing, stretch in the longitudinal direction and finally are torn apart. The end of the strength of the vascular bundle also signifies the end of the pressure exerted against the meristema cells; as a result of this, the cells to be exposed assume a tube shape and the leaf, which no longer is connected with the stem by any strand tissue, is removed from its point of insertion. In the basal tissue, the separation of that which is to be cast off from that which remains behind occurs in the middle lamella through the uneven growth of the participating elements. It was observed repeatedly -- and we mentioned earlier in connection with *Cinnamomum Reinwardti* -- that even before the formation of the tube cells the leaf can be caused to separate by means of the application of a small amount of force whereby the meristema cells fall apart with smooth walls; this can be tied in with the fact that the intercellular substance is loosened as a result of chemical agents; but this assumption is not absolutely inevitable here.

The process is somewhat similar in the case of *Laurus canariensis*, *Cinnamomum Reinwardti*, *Cinnamomum Camphora*; we also observed tube cell formation along the fresh defoliation wound in *Cinnamomum albiflorum*, *Apollonias canariensis*, perhaps *Ficus stipulata*, as well as in several citrus species and *Pilocarpus pennatifolius*; but we did not have the necessary material for any more detailed investigation.

#### IV. Dependence of Form of Absciss Layer on Internal and External Factors

In the introduction to this work on defoliation Tison noted that he had studied leaf separation only on trees growing out in the open at the time of their natural defoliation in the autumn, in other words, he did not study this in potted hothouse plants or in plants in which defoliation was brought about by some artificial means. The processes described by Tison thus are certainly purely physiological. But in order to get a complete picture of the anatomy and physiology of leaf fall, it would be desirable to investigate the same plants at different times, when leaf separation occurs as one of the physiological types described by Wiesner (see Bibliography, Wiesner, III-VI).

The observations, reported below, on evergreen plants seem to make it appear possible that the absciss layer, even in the case of summer-green plants, differs in accordance with the causes leading to the dropping of the leaf; Kuster (L.c., page 189, Note 3) asked the question as to "whether the separation tissue, developing along mutilated leaves and under the

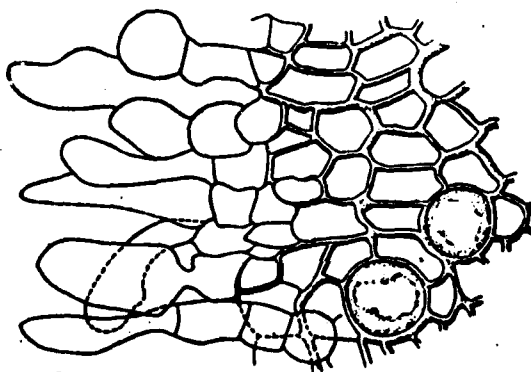
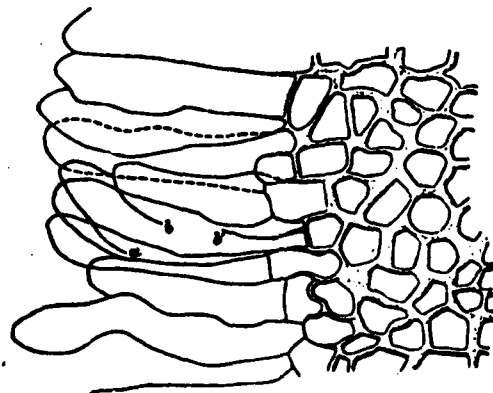
effect of moist air, is identical with the normal tissue"; this question as a result seems to be quite justified.

(Mohl (II) partly answered this question: when we force the leaf fall in an absolutely moist space or room, we never have periderm formation; the cells do not take on a brownish color, as in autumn. But the process of separation is modified due to the effect of frost (Mohl, I): the cell content forms irregular balls and the membranes are partly torn up.)

Wiesner's numerous investigations taught us to recognize leaf fall in its various subdivisions, such as autumn, summer, sprouting, heat, and frost leaf fall. Plants with sprouting leaf fall lose leaves also outside of this type of leaf fall, and in this case it is primarily the older leaves that drop; this process, which is based on internal factors, we will call hereafter "sporadic defoliation or leaf fall." The difference in the cause is also expressed in the difference in the separation tissue. In sporadic defoliation of *Laurus nobilis* (in the winter this was observed on potted trees about 2 m high which stood in a cold but frost-free room), the fresh defoliation wound was covered with long tubes or hoses (Figure 6) which were either sitting directly on top of the normal parenchyma or by means of a second intermediate thin-wall cell layer (Figure 7); on the other hand, the separation tissue during sprouting defoliation (observed in June and July partly on the same trees, now standing in the open) consisted of several cell layers of which the exposed cell layer consisted of short tubes (see Figure 2, page 383, Ost. bot. Zeitung, 1906).

In *Cinnamomum Reinwardti* (a cold-house plant, more than 2 m high) the total volume of the sporadically fallen leaves (early winter) was roughly the same as the volume of leaves that had fallen as a result of sprouting defoliation (February). The latter was not as clearly pronounced as in *Laurus nobilis* especially since it was not separated from sporadic defoliation by a long interval of time. While the numerous fresh defoliation wounds of sporadically fallen leaves investigated revealed enlarged cells with knob-like swollen ends on the exposed surfaces (Figure 8), we found that individual leaves which were investigated early in February, when the sprouting began, revealed long tubes which had been formed already before the leaf cell (Figure 9).

(This was quite striking; as we explained in Section III, the tube cells (spikes or knobs) of *Cinnamomum Reinwardti* are formed just before the fall; but here we are dealing only with leaves that have fallen sporadically.)



Figures 6 and 7. Enlarged 200 X. *Laurus nobilis*, sporadic leaf fall. Longitudinal section through fresh leaf fall wound. The type shown in Figure 6 is the more frequent one here. a, b, b' -- cell walls torn during preparation.

One rather noteworthy exception to the rule -- to the effect that the absciss layer always appears at a place which is quite specific for each plant, even though it does not differ in any way from the environment (Mohl, I), usually that is, prior to the time when the changes leading to leaf separation occur -- is represented by *Cinnamomum Reinwardti* in that individual leaves, sometimes several on the same sprout, develop their absciss layer sometimes not at the normal place but somewhat higher, at times about 2 mm and more; this of course is a process which is quite rare; (it was observed about 10 times on one tree over a period of two winters). The tube cells were similar to those developing on the normal spot but they were less rounded at the end than the normally developing tube cells; more specifically they were -- in the only case investigated during sprouting -- considerably longer than otherwise.

We get a thicker absciss layer when cut-off *Laurus* sprouts are caused to drop the leaf stems in an absolutely moist room by means of the removal of blades. The epidermis is torn up in the form of a clearly gaping ring-shaped furrow and on the longitudinal section we can then, after leaf separation, see the free ends of the tube cells in two floors or levels, one above the other (Figure 1 on page 382 of *Ost. bot. Zeitung* 1906). The opposite ends often would appear to be on the same level so that, as the result of the growth of the longer tubes, the leaf stem tissue is replaced by the shorter ones.

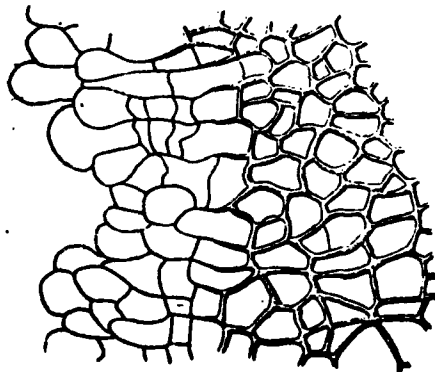
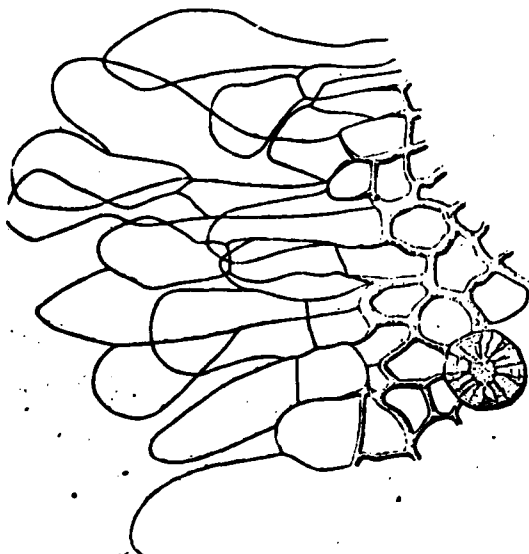


Figure 8. Enlarged 200 X. *Cinnamomum Reinwardti*. Longitudinal section through fresh defoliation wound (sporadic defoliation); short tube-shaped cells with ends widened in form of bulb.



**Figure 9.** Enlarged 200 X. *Cinnamomum Reinwardti*. Tube cell formation during sprouting. (The leaf was brown and dropped on contact; this means that subsequent growth of the tubes is impossible here.)

In the case of *Evonymus japonica*, a very great multiplicity of elements was observed in the absciss layer. The formation of the absciss layer starts from the middle of the absciss zone which consists of small-cell tissue (Figures 10 and 11). Sprouts which had been cut off and which stood in water with their cutting surface dropped all leaves in dry air within a few days (in the winter about 7 days and in the summer about 5 days). In the winter the mechanism consisted of a strong turgescence of round cells while in 3 experiments conducted in the summer, the cells, which came off individually or in groups, had a trapezoidal shape in an optical cross section; this maceration was probably responsible for the break-up of the cell combination. In an identical experiment, conducted in artificially dried air (I, 20), the cells were irregularly stretched along the longitudinal line; they had thin walls; some of them had a long protrusion at one end or on one side into which the lumen extended; the cause of the general isolation here would likewise appear to be maceration.



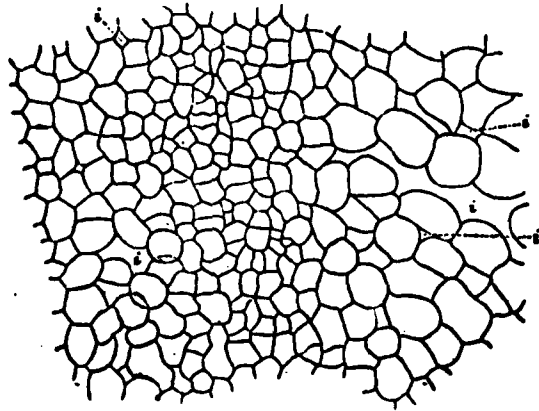


Figure 10. Enlarged 200 X. *Evonymus japonica*, normal leaf joint with absciss zone. i -- Intercellular spaces.

It is very difficult or entirely impossible to achieve defoliation in an (absolute) moist room (this is due to the fact that the sprout eventually strikes roots (in the winter); when the sprouting time as such comes, this leads to leaf fall (II, 3, 4)). But this can be achieved easily if we allow one factor, which promotes defoliation, to act here likewise (in this plant, darkness helps very little in defoliation, especially in the winter (I, 17; II, 4)); this factor is drying (here the sprout, which is not dipped into water with the cutting surface, is simply left to stand in the air) (II, 7), as well as withdrawal of carbonic acid (III, 6; VIII, 2), and increased temperature (VIII, 5); in all three cases it takes only a relatively short period of time (1/2 - 2 weeks) for defoliation; defoliation here takes place by means of the tube cell mechanism.

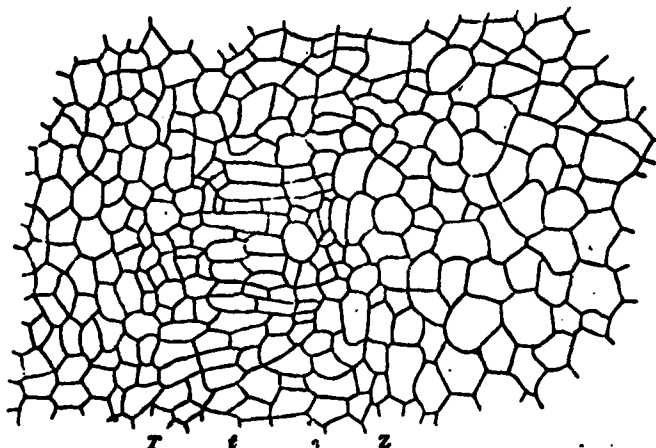


Figure 11. Enlarged 200 X. *Evonymus japonica*, development of absciss layer (forcing through heat -- material taken from VIII, 5c). TZ -- absciss zone; ts -- absciss layer.

#### V. Some Experiments on Forced Leaf Separation

Citrus species have a leaf stem which is set off not only against the axis but also, very clearly, against the leaf blade; the separation can take place in either of the two joints. A potted specimen of *Citrus medica*, in connection with leaf fall on the upper joint, revealed cells which were swollen up in bulb-form along the exposed leaf stem surface while when the leaf fell off on the lower joint both exposed surfaces were covered with isolated round cells. It was noted that, in case of simultaneous drop of several leaves, either all of them or almost all of them were separated on the upper or all of them were separated on the lower joint, in other words, that the leaf fall was not anywhere near uniformly distributed over both joints but that one of them was clearly favored. Whether, in this repeatedly observed phenomenon, we are dealing with a rather insignificant coincidence or whether this is an adaptation to two different factors leading to defoliation --one which acts primarily on the upper while the other acts primarily on the lower joint -- could not be determined because the material was not available. Similarly, only one of the experiments mentioned later on could be conducted in connection with *Citrus Aurantium*. During the separation of the leaf along its base, from the stem, the vascular bundles were separated in both plants with smooth walls, without becoming torn; they were not lignified at this point. In the potted sample the leaf stems, which had remained behind on the sprout, likewise fell off after some time.

That the absciss layer is an adaptation and not an organization characteristic follows not only from the ease with which it is capable of changes but also from the fact, clearly to be observed in Tison, that entirely different species have an anatomically entirely identical structure of the absciss layer and vice versa; this is also indicated on the basis of the widely different physiological behavior of closely related species. *Cinnamomum Reinwardti*, for instance, is very resistant against humid air; *Cinnamomum Sieboldi*, on the other hand, lost most of its leaves (I, 19) in an absolutely moist room already after a few days -- in this case, however, by means of the round-cell mechanism. It was rather odd to note the difference in the behavior of *Evonymus japonica* and *Ev. Schottii* in combination with the greatest possible and the smallest possible transpiration. The end of the 6-day experiment (I, 20, 21) is illustrated by the following table:

	<u>Room as dry as possible</u>	<u>Absolutely moist room</u>
<i>Ev. japonica</i> ...	Leaves dried out on sprout, one leaf fell off by itself (maceration mechanism)	unchanged (which is quite natural according to I, 17)
<i>Ev. Schottii</i> ...	Still fresh	Leaves fallen off (maceration mechanism)

The macerated cells of *Evonymus Schottii* were extraordinarily long and thin-wall; the cells of *Evonymus japonica* were described in the preceding section. Two parallel experiments, launched in the same fashion, involving *Citrus Aurantium* (I, 24) revealed a high sensitivity against both extremes: the drying-out was complete on the 3rd day, defoliation began in an absolutely moist room on the 4th day while the still firmly adhering leaves usually fell off upon contact, more specifically, roughly evenly along the upper and the lower articulation; similarly, we also observed the tube-cell mechanism along both joints; the tubes of the lower absciss layer were particularly large and contained starch while those in the upper layer, with the bulb shape, did not have any starch. *Laurus nobilis* dried out in the middle of the 2nd week (I, 22) while *Aucuba japonica* was still alive, but completely limp (I, 23) on the 12th day when the experiment was broken off.

In a carbonic acid-free room, according to Furlani ("On the Effect of Carbonic Acid on Defoliation," *Ost. bot. Zeit.*, 1906), only those leaves will fall off whose insertion points are still in the meristematic condition. In *Cinnamomum Reinwardti* (III, 7) we indeed observed only the fall of younger leaves while older ones, even those which were already closer to natural defoliation and which had become yellowish or brownish, remained strong. The tube cells had the shape of short cylinders. In the 2nd experiment (VIII, 7) in the carbonic-acid-poor room, an apparently completely adult leaf fell off during the 5th week in a completely green state.

The separation tissue consisted of long tubes which however did not -- as always in the case of the spontaneous and experimentally produced leaf separation -- remained firmly in contact with the foundation but which also in large numbers covered the leaf wound completely freely (Figure 12).

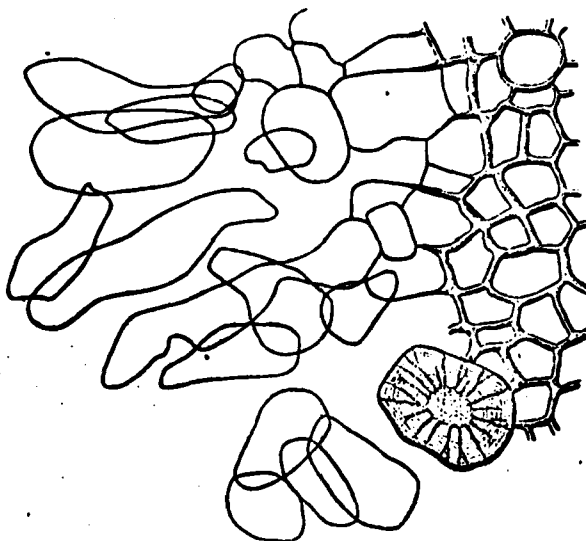


Figure 12. Enlarged 200 X. *Cinnamomum Reinwardti*, maceration of separating cells in CO<sub>2</sub>-poor room.

In *Evonymus japonica*, all leaves fell off (VIII, 2); the tube cells were bulb-shaped and, as a result of longer stay in the carbonic acid-free room, appeared to have become longer; here, likewise, we had a maceration of the separation cells and some of them assumed forms similar to those found in the experiment in the driest possible room (I, 20).

*Buxus sempervirens* lost all of its leaves (III, 6, b); the separation took place by means of long cells arranged in two layers, one on top of the other, which did not reveal any separation from each other along their longitudinal walls and which formed a hyperplastic tissue mass that exceeded the normal diameter of the leaf base in the radial direction.

The influence of increased temperature was investigated in the heat cabinet in the dark, mostly in moist air. At about 30° C (the temperature from time to time rose by 1-3°) *Evonymus japonica* took 4 days until full defoliation (VIII, 5), *Stiftia chrysantha* took 3 days (VIII, 8), *Nerium Oleander* took 9 days (VIII, 16; IX, 1), *Eugenia Ugni* took 6 days (VIII, 12). At 40° C *Evonymus japonica* did not reveal any defoliation at all; instead, the leaves dried out on the stem within 5 days, regardless of whether the air in the heat cabinet was moist (II, 8) or dry (III, 2).

Sprouts of *Taxus baccata* lost individual needles at around 30° C (IX, 2) already toward the end of the 1st week. After 10 days most of them were so loose that they could be knocked off by means of a slight bend downward. Only very few of them fell spontaneously; when the experiment had to be discontinued in the middle of the 3rd week, some of the needles, especially those along the main sprouts, had become brownish toward the tip while the needles further down and those in the side sprout remained green. In both groups of needles, it was mostly enough to cause a minor impact or a gentle touch, in some of them, of course, a somewhat stronger bending action, in order to separate them; only very few of them had a firm hold on the stem. Longitudinal sections through the leaf joint revealed a thin-wall separation tissue (Figure 13); but this extended only from the leaf groove strand to the upper epidermis; an older stage, with an absciss layer permeating the entire needle base, was not investigated; similarly, the process of separation could not yet be observed.

At ordinary room temperature *Taxus* sprouts remained unchanged both in the absolutely moist room and in a carbonic acid-free room throughout 3 weeks.

The separation mechanism at increased temperature was discussed earlier for the case of *Evonymus japonica*. In the only experiments made with *Stiftia* and *Eugenia*, the separation cells, emerging from the absciss zone in the case of the former, were more or less rounded along the exposed side; but we did not have any complete isolation almost anywhere along here; it was rather interesting to note the particularly small thickness of the separation tissue.

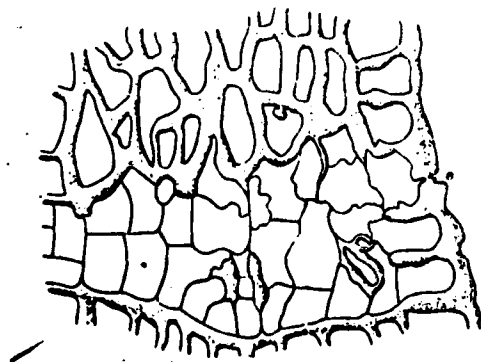


Figure 13. Enlarged 300 X. *Taxus baccata*, formation of an absciss layer through the effect of higher temperature. e -- beginning tear epidermis.

In Nerium the leaf base was rather dry while the free leaf fall wound was covered with a thick layer of large, heavily turgescient, thin-wall cells so that the difference in the tension was probably the cause for separation here. (For the defoliation due to turgescence of the cells remaining behind on the leaf cushion, see Wiesner, "On Frost Defoliation With Some Comments on the Mechanics of Leaf Separation, Type c.")

Other leaves involved in the same experiment revealed a similar behavior as described under Buxus (Figure 14). (This mechanism here would appear to be the same as in the case at hand.)

In Eupatorium adenophorum, whose inability to get rid of its leaves was explained biologically by Wiesner (Die biologische Bedeutung des Laubfalles (The Biological Significance of Leaf Fall), Section 8) and from this the author concluded that leaf separation cannot be forced through any artificial means; in this case of Eupatorium adenophorum the factors which normally produced this leaf separation (absolutely moist room, CO<sub>2</sub>-poor room, increased temperature) merely led to the result that the cut-off sprout grew very actively in a longitudinal direction; as new leaves developed, the old ones died off and dried out entirely, just as in nature, on the stem; even when the water in which the sprouts stood, extended above the lowest leaves, we found that these leaves shriveled up, accompanied by discoloration and one might almost say that they likewise dried out.

## VI. Separation of Axis Formations

When Cinnamomum Reinwardti casts off axillary buds, this happens under the same anatomical conditions as during defoliation: formation of enlarged, thin-wall cells with ends broadened in bulb form. Offshoots were never observed and injured branches simply dried out. (Hohnel, "On the Separation Process of the Branches of Some Wooden Plants and Its Anatomic Causes -- Further Investigations on the Separation Branches of Lignified Branches," Mitt. d. forstl. Versuchswesens für Österreich, (Bulletin of the Forestry Research Department for Austria), Vol I, No 3, and Vol II, No 2.)

Stumps of branches likewise dried up until they reached a certain place which was sharply delimited against the living tissue -- but they did not drop; the same happened in the case of Laurus nobilis, Citrus Aurantium and C. medica.



Figure 14. Enlarged 40 X. *Nerium Oleander*, defoliation forced through heat (the leaf was not separated until cutting time, the separation tissue thus was certainly hyperplastic even before its exposure). A -- axillary bud; BCDE -- free edge of defoliation wound, abcd -- boundary of normal basal tissue; def -- part of hyperplastic separation tissue; ghx, lay, kz -- direction of cell strands in normal basal tissue; SS' -- vascular bundle.

In *Evonymus japonica* various types of separation of axillary formations were observed. Two small potted trees, which began to sprout during the winter (presumably because they were kept in a warm area during transportation), dropped the freshly sprouted branches in the cold-house after sprouting defoliation, in other words, both the main sprouts and the side sprouts, more specifically, in the "branch cushion" (Hohnel, I). But the process should not be considered as an offshoot because we are dealing here with very youthful, just sprouted organs. The separation mechanism consisted mainly of maceration: after the

bursting of the epidermis, the crack was widened due to the accumulation of isolated, very thin-wall, hypertrophic cells of varying shape, mostly spherical, however. The cause here would appear to reside in the excessive moisture content in the air; later on, numerous hyperhydric bark proliferations developed.

Along with the cut-off sprouts of *Evonymus japonica*, the ramifications, like the leaves, are separated only over larger periods of time as a result of the simple stay in a dry room environment. The separation zone in the sense of Hohnel (I) is present insofar as the strand [vascular] tissue of the side sprout does not form any compact wood cylinders within the branch cushion but is instead dissolved into numerous wood strands, between which we have nonlignified (parenchymatic) tissue.

The separation cells of the parenchyma are long-drawn out and fall apart through maceration; their proliferating force, however, as a rule is not enough to cancel out completely the connection of the piece to be separated with the main sprout; instead, it is just enough to loosen it quite considerably; very often it takes an external although very slight push in order to break off the wood strands, of course, to the extent that they are still intact.

A rather strange separation process occurs after defoliation in dry air on sprouts of *Evonymus japonica* if we decapitate them. The cutting surface begins to dry out while, several days later, right above the next lower axillary buds, we can see a ring-shaped swelling which becomes more and more pronounced until the bark all around breaks up. The gaping crack does not go around the sprout simply in a circular fashion; instead it is somewhat above the axillary buds on both sides while, in the meantime, it pulls the sprout lower down (Plate, Figure A). If the sprout was kept in an absolute moist room after decapitation, then, in addition to the simply circular break up, we also had radial tears running upward so that the bark at the boundary between the two internodes is subdivided into a number of radial lobes protruding away from the axis (Plate, Figure D). Next we have a large number of all-round isolated, hypertrophic, viable cells welling up out of the cracks, especially during the experiment in moist air (Plate, Figure B, a'; Figure C, c). For reasons which I was unable to determine completely, the break-out or break-up in the dry room sometimes took on a different form in that the formation of the circular furrow did not take place; instead, about 5 mm and more, from the node upward, there were shallow cracks which spread in a radial fashion in the swollen bark



(Plate, Figure E). Similarly, however, it happened very rarely that we had a ring-shaped break-out immediately under the point of decapitation; sometimes this break-up later on turned into a radial one, heading downward, by means of a number of cracks and tears (Plate, Figure F); in both cases there was no isolation of large masses of cells. Several sprouts of both of the little potted trees behaved similarly after defoliation; the tip of the sprout, with the top-most leaves, had been damaged on these trees during the winter and had dried out. On a longitudinal section through one of these break-ups burst sections we can recognize that the bark tissue is heavily proliferated and loosened while the xylem was unchanged; the marrow runs through a zone which extends in a lateral direction and which is sharply delimited against the remaining bright-green tissue, both up and down, by virtue of its darker color; under the microscope, this zone reveals a denser content and cell subdivision and must thus be termed an absciss layer.

Of course, there could be no real dropping of the internodium because of the complete lignification of the homogeneous vascular tissue; instead, the xylem dries out along with the marrow and bark, to the end. (See Hohnel, I, "Absence of off-shoot and presence of dried branch in *Thuja occidentalis* when separation tissue -- nothing more than a lateral cork lamella was observed, by the way -- is abnormally higher than the abscess zone"; see also Hohnel, II, page 251, "Absence of the absciss zone in wood while it is present along with the absciss layer in the marrow and bark.") A burst-up, which develops on the youthful tissue after injury (drying out) of the sprout tip, however can lead to complete separation (Plate, Figures A and B).

The swelling of the burst-up occurs as a result of increased growth of the bark parenchyma cells primarily in a radial direction (something similar here is represented by the bark proliferations described by Kuster and by the hypertrophies of the bark cells in a radial direction which were observed in connection with the off-shoots noted by Hohnel (I); in this case, by the way, it is the cells that are located most peripherally which are macerated and which break up all around.

The condition for the occurrence of a break-up or burst-up is the absence (or the nonfunctioning) of the next higher axillary bud tear. If, in a decapitation experiment, the axillary buds which are now uppermost were removed simultaneously then we do get a burst-up on their level but a short time afterwards we could observe the beginning of the formation of a second burst-up on the next-lower bud node. Adult, organically defoliated branches, in which the terminal bud and several axillary bud pairs had been extirpated, formed the burst-

up on the level of the first bud pair obtained.

#### VII. Polarity Phenomena During Separation Processes

So far it has been observed without exception that, in case of separation by means of tube cell mechanism, the cell hypertrophy occurs always only on the sprout side; this points toward a puller contradiction between the tissues of the stipule. On the upper joint of the Citrus species, the tube cells could be found only on the leaf stem, in other words, once again on the basiscopic side. When the defoliated, decapitated sprouts were placed in the water partly upright and partly upside down (or when they were put in moistened cotton), then the exposed cutting surfaces were subject to the effects of drying in the same fashion; but it is only the ends of the upright sprouts which dried out and which shrank very considerably while those that had been stood up upside down, other than along the cutting surface itself, did not reveal any major drying phenomena. Even in an absolutely moist or humid room, we were able to note a difference in the drying of the cutting surfaces, insofar as the sprouts that were placed upside down remained fresh for a longer time. The burst-up developed only in the upright sprouts while the absciss layer in the marrow developed also in those that stood upside down, however this time on the lower end which represents the apical pole. In no case did we observe a burst-up on the upside down sprouts along the upward pointed basiscopic side, although they were exposed to the same conditions as the acroscopic ends of those that stood upright.

#### VIII. General and Comparative Considerations on Separation Processes

In view of the great similarity of organ separations in the vegetable and animal kingdoms, in biological terms, and in view of the difference of plant and animal organs, in terms of structure and functions, it might be interesting to find out more about the histological structure and the development history of those tissues which facilitate organ separation as well as the mechanism of separation in the case of animals. As far as I know, there are just about no investigations on this topic. The only thing on which we have a careful study is the replacement of teeth in mammals; the tooth pouch, which contains the young tooth, proliferates under the old tooth, growing upward, and gradually resorbs a large portion of the tooth substance. As far as the replacement of hair is concerned, we do have a number of observations but these do not agree with each other. (All we have here, by the way, involves the "sporadic" change or replacement of human hair; the histo-

logical conditions of the periodic hair replacement of animals in the temperate zones at the beginning of spring as well as the replacement of hair connected with the growing and shedding of the white winter skin of polar animals do not seem to have been investigated; the same applies to the molting of birds.) Furthermore, we have some knowledge of the mechanism of separation from the subject of autotomy in lobsters; the extremity, which is either caught somewhere or which has been grasped by an enemy is torn off by means of a muscle contraction at a preformed place ("absciss zone"); this solves a second problem which probably does not turn up in any other group of organisms: the immediate closing of the wound; since the heart is in open communication with the body cavity and since the latter is placed in contact with the outside world due to the injury to the extremity, the animal cannot wait until a wound-closing tissue has developed; instead the outflow of blood must be prevented by means of the immediate blocking of the opening; this is done by means of a membrane which, along with the autotomy, closes the wound (Dungern, Dr E. Freiherr v., Die Antikörper, etc. (The Antibodies, etc), Jena, 1903, page 72). The "absciss zone" is also supposed to exist in the tail vertebra of the lizard; but it seems that no one has investigated the way in which the separation of the shaft part takes place.

The table below shows a list of various organ separation processes arranged into 3 groups from biological viewpoints. In the first group we have those processes in which the organ to be dropped is in the process of dying out for internal reasons or is already dead; in the case of the separation processes in the third group, which likewise take place because of internal factors, we are dealing with living parts or with parts which live for a longer time or which remain alive permanently; they constitute new organisms or they lead to the formation of such new organisms. The second group contains a number of examples for the separation of organs in a completely live state which, however, must perish soon because they are viable alone.

#### I. Separation of Dead or Dying Organs

- |  |  |
|--|--|
| 1. Periodic leaf fall (autumn and sprouting defoliation) | Periodic hair change or replacement in mammals, tooth replacement; periodic dropping of apoplasmas in mammals (horns); autumn molting of birds |
|--|--|

- |   |  |
|---|--|
| 2. Separation of bark (rind, crust)   | Dropping of apoplasmas in order to facilitate the further growth of the enclosed body (shedding in crustacea, metamorphosis of insects)  |
| 3. Dropping of male blossoms after anthesis, same for perianthium; female or hermaphrodite flowers or blossoms, in case there was no fructification | Spring molting of birds for growing of mating feathers (to the extent that the formation of this mating dress does not reflect a mere pigmentum transformation; dropping of wings by some insects, after mating) |
| 4. Dropping of branches that were injured or are less viable for other reasons  | Rejection of injured extremities in arthropods   |

## II. Separation of Living Organs Which Cannot Live Alone

- |   |  |
|---|--|
| 5. Dropping of green living leaves due to outside effects (for instance due to sudden frost, see Wiesner VII, Section 3, or due to sudden absorption of water after severe drought, see Wiesner II, page 87 (Azalea)) | Breaking off of lizard tail; autotomy of lobsters and spiders (in the latter we have a chitinless ring between the coxa and trochanter which represents the "absciss zone", see Friedrich, P., "Regeneration of Legs and Autotomy in Spiders," <u>Archiv fur Entwicklungsmechanik</u> (Archives of Development Mechanics), Vo 20, 1906; the process of separation here is similar to that in the case of the lobster |
|---|--|

## III. Separation of Live Parts Which Perform Certain Functions After Separation

- |   |  |
|---|--|
| 6. Dropping of leaves for the purpose of vegetative multiplication (for instance, Bryophyllum); dropping of breeding buds | Dropping of statoblasts from funiculus (sweet-water bryozoa) |
| 7.  | Separation of Ephyra from Strobila (Scyphomedusa)            |

8. Lateral division of worms. Scolecida: among the turbellaria (some Triclada and Microstomides). Annelidae: among the Chaetopoda (and more specifically, Polychaetae) (in Syllidae, like Autolytus)
9. Separation of male blossom of Vallisneria Separation of epitoke parts (containing the sex products) of Eunice viridis (Palolo worm); Separation of hectocotylus of cephalopoda (in order to put the spermatophores into the mantle cavity of a female individual)

As we can see from the table, the processes in the first group serve further development (growth) of the organism or for the removal of useless organs; the processes in the second group arise as a result of outside factors and are partly protective devices [defense mechanism] (in the case of animals); the processes in the third group, however, are used for asexual partly (9) also sexual propagation [reproduction].

This table of course is far from complete; it is merely intended to list a number of separation processes and to alert the reader to the parallelism in the animal and vegetable kingdom.

#### IX. Summary

1. We can currently distinguish the following mechanisms on the basis of the direct cause which predominantly brings about the separation of organs: I. round-cell mechanism (Mohl); II. dissolution mechanism (Tison); III. maceration mechanism (Wiesner, Kubart); IV. Turgescence mechanism (Wiesner); V. Tube-cell mechanism; VI. Hard-cell mechanism (Bretfeld, Molisch).

2. Both the round-cell and the maceration mechanism develop as a result of the joint effect of increased turgescence and dissolution of the intercellular substance and differ from each other in that, in the former, the dissolution processes are quite secondary in significance compared to the turgescence while in the latter the process of maceration also plays a very outstanding role and the isolated cells can have widely differing shapes.

3. The formation of the absciss layer leading to the dissolution and to the tube-cell mechanism is initiated by a

change in the structure of the thickening layers; in the first of these, this is followed by a dissolution of the cellulose until the participating cells are delimited only by a thin membrane corresponding to the innermost portion of the original wall; in the latter, however, the membrane thinning occurs as a result of the growth of the original cells and the meristema formation as a result of which the separation cells /absciss cells/ are enclosed, not as in the first case, merely along the side to be exposed, but all around; they are enclosed here by thin membranes. They are separated from each other primarily as a result of uneven growth, especially in the longitudinal direction.

4. The turgescence mechanism leads to separation as a result of the shift of the thin-wall, heavily turgescient cells along the less turgescient or shrinking cells of the piece to be dropped. (In two experiments we observed a rather significant hyperplasia of the absciss layer with a volume increase also in the lateral direction which contributed to the separation of the less turgescient, not hyperplastic tissue parts of the leaf.) The separation along the boundary of two cell layers of differing nature is a common feature in this mechanism with the hard-cell mechanism.

5. The anatomic nature of the absciss layer is not always the same in the same species; instead it varies as a result of the influence of internal and external factors; the separation mechanism as such can also change.

Here I would like to point out a contradiction in literature. Mohl lists *Aristolochia Sipho* among the plants which reveal a clear meristema formation; according to Tison, however, the absciss tissue consists of a layer of cells which become longer while their membranes become thinner in the middle and finally are torn at the thinnest point. Despite the extreme care taken in Tison's investigation -- as indicated in the introduction -- I think that an error here is not entirely impossible. In a comparison between Tison's illustration and a developing absciss layer of the tube cell mechanism we will not be able to deny a certain similarity in the wall thinning. I would like to note that I cut the leaf joints of yellowed and browned leaves of *Cinnamomum Reinwardti* for many weeks on end without finding any absciss layer; on occasion, however, when the leaf, during the attempt at cutting it or during the cutting, would tear off, I was able to see the lumens open and this of course made me think of a strength reduction due to a change in the nature of the membrane -- a strength reduction which perhaps also facilitate leaf separation in nature; later on this assumption turned out to have been wrong. But

this does not mean that I am trying to say that this tearing mechanism does not actually occur in fact. According to the experiences reported in Section IV, it would be possible for some kind of outside factor (we might of course not assume an internal factor here because both of the investigations were made in autumn) might be made responsible for the difference in the observations; this outside factor might perhaps be the difference in the climate. In order to find out in which way the climate influences the absciss layer, we might find suitable rose plants which -- just like *Ligustrum vulgare* (Wiesner, Biolog. Bed. d. Laubf. (Biological Conditions of Defoliation), Chapter 11), can be summer-green or evergreen (the cherry tree in Ceylon and the birch tree on Madeira are evergreen; Wiesner, Biologie (Biology), 1902, page 82); here we might also mention some of the evergreen deciduous trees which belong to a species in which we also have summer-green ones, such as *Ligustrum Kellermanianum* in comparison to *L. vulgare*, *Berberis illicifolium* in comparison to *B. vulgaris*, *Prunum Laurocerasus* in comparison to the summer-green *Prunus* species; it might be worth while here to investigate identical species at different geographic latitudes.

6. The mechanisms are not sharply delineated against each other; instead they are tied in at certain transition points.

In addition, the work contains special data on an abnormal position of the absciss layer (*Cinnamomum Reinwardti*) on the separation of axis formations, on polarity phenomena and a number of physiological observations, as well as a comparative table of various organ separation processes which also takes into consideration the events in the animal kingdom.

I owe the motive behind this work to the kindness of my very revered teacher, Professor Hofrat Court Counsellor Dr J. Wiesner and I want to express my sincere appreciation to him at this point. Originally my job here was merely to find out whether it is correct to say that *Cinnamomum Reinwardti* drops its leaves not through an organic process but rather that the leaf stems, which by nature are not very strong anyway, become even more brittle at the time of leaf fall and that they break off, due to outside forces, at any point along the leaf stem, accompanied by the tearing of the cell walls. The discovery of the tube cells and the tracing of the development of the axis layer, which I managed to achieve many months later, provided for the impetus for further studies in this field.

I also want to thank Private Docent Dr Karl Linsbauer for his interest in my investigations and for the support he

gave me in my work.

X. Appendix (Excerpt from Record of Experiments)

Unless otherwise indicated, the experiments were conducted with sprouts which had been cut off and whose cutting surface had been placed in water; this was done in the experimentation room of the plant physiology institute; this room had the usual atmosphere found in any living room, in other words, it had a very low humidity (this is why I always used the term "dry room air" in the text); in the summer, moreover, the room was very heavily heated by the sun. The following data are intended, among other things, to help discover any deviations -- in case someone wanted to check on the experimental results -- which might be due to the fact that we are dealing with different vegetation periods in connection with the plants used (for instance, when we make an experiment at a different season of the year) and to avoid any errors resulting from the neglect of this circumstance.

Abbreviations: A -- absolute; f -- moist, humid; r -- room; Ev/onymus/ Jap/onica/; W -- heat; S -- cabinet.

Series Experiment

- |   |    |  |
|---|----|--|
| I | 17 | Ev. jap. AfR, cold-house, in the dark, 11 May - 11 June 1906; Microscopic report: the fresh defoliation wounds are covered with cells which are partly long-drawn out, which are not much enlarged, and which are moving apart (cf., IV, 7)(dissolution mechanism?).   |
| I | 19 | Cinnamomum Sieboldi, AfR, cold-house; experiment begun on 20 June 1906.  |
| I | 20 | Ev. jap. Investigation of the effect of driest possible air, 20-26 June 1906; the cut-off sprouts were placed in a glass of water with their cutting surface; the surface of the water in the glass was covered with a thin oil layer in order to delay evaporation; the glass stood in a large crystallization cup filled with $\text{CaCl}_2$ ; this entire set-up stood on a matted glass plate on which a glass bell had been placed, providing hermetic sealing and which had been ground; the air space was always dry because the transpiration water absorbed by the $\text{CaCl}_2$ . |



One leaf fell off spontaneously; the others dried out, in which connection the shrinking stem contracted so considerably towards its starting or base surface that a portion of that surface was exposed in the form of a wound surface. The absciss cells moved apart as a result of maceration (for their shape, see above).

- |    |         |  |
|----|---------|--|
| I  | 21      | Ev. Schottii, 20-26 June 1906; behavior in driest possible (same experiment as under I, 20) and in AfR.  |
| I  | 22      | Laurus nobilis, driest possible room (same as under I, 20); experiment started on 20 June 1906.  |
| I  | 23      | Aucuba japonica, 20 June-2 July 1906, driest possible room (I, 20).  |
| I  | 24      | Citrus Aurantium, experiment in AfR and driest possible R; experiment started on 22 June 1906.   |
|    | 25 June | Dry R., completely dried out.  |
|    | 26 June | AfR: one leaf fell off spontaneously on the upper joint, others fell off upon contact partly on the upper and partly on the lower joint; for microscopic report, see above.  |
|    | 28 June | two leaves fell off spontaneously on upper joint; the vascular bundles appear to have moved apart and broken up for the most part and perhaps entirely without any tearing.  |
|    |         | Experiment discontinued.   |
| II | 3       | Ev. jap. AfR, cold-house, under light.<br>From 25 October 1906 until 1 March 1907 no change (except for the development of roots in January); sprouting began at the end of January; the spontaneous dropping of 2 leaves on 1 March is perhaps a result of sprouting; on 12 March, 4 more leaves dropped. Experiment discontinued.  |
| II | 4       | Ev. jap. AfR, cold-house, in dark.<br>From 25 October 1906-24 January 1907, no change; on last here the lowest leaf, colored yellowish on the base, broke off upon contact. (Three root beginnings which later on died off again.)<br>28 January -- spontaneous dropping of one leaf which had turned yellow and whose stem was still standing in the water.<br>15 February -- 4 leaves dropped; |

- 1 March -- 2 leaves dropped;  
 12 March -- 2 leaves dropped.  
 Experiment discontinued. (There was no sprouting.)
- II. 7 Ev. jap. Hothouse, simply set up (on the sand); experiment began on 25 October 1906.  
 31 October -- 1 leaf dropped spontaneously, 2 dropped as a result of shaking /impact/.  
 2 November -- defoliation progresses acropetally.  
 6 November -- sprouts completely defoliated with exception of 2 bushel or bunch, each, at the tip.
- II. 8 Ev. jap. WS 40° C. Air moist (bottom of WS under water). 5-10 November 1906.  
 7 November -- terminal bud of main sprout thickly swollen, that on side sprout less swollen but still swollen to double the size of the axillary buds.  
 8 November -- bud growth discontinued again.  
 9 November -- buds turning brown; surface of leaves covered with numerous, lighter-colored depressions.  
 10 November -- leaves dried out.
- III. 2 Ev. jap. WS 40° C. Air dry (water, in which sprouts were placed, was covered with oil layer); 16-21 November 1906.
- III. 6 a) Ev. jap  
 b) Buxus sempervirens } in CO<sub>2</sub>-free air (absorption of CO<sub>2</sub> by concentrated KOH).  
 17 November 1906 -- Start of experiment.  
 25 November 1906 -- (a) start of leaf fall.  
 4 December -- (b) the ring furrow, which had been observed for some time on many leaves, has widened into a deep crack which runs through all tissues so that leaves adhere only along a small piece of the lower epidermis. Many of the other leaves also fell off upon contact or shaking.
- III. 7 Cinnamomum Reinwardti in CO<sub>2</sub>-free air (absorption of CO<sub>2</sub> by concentrated KOH); experiment started on 7 December 1906.  
 A portion of the leaves was brown. During the 3rd week the green leaves were separated and this includes even those which, judging by their size, were adult (it was unable to determine whether any meristematic tissue was left in the leaf joints); but the brown leaves were still firmly connected during the next week.

- IV 7 Ev. jap.  
 21 November 1906 -- start of experiment, dry room air.  
 24 November -- transfer to cold-house.  
 5 December -- defoliation.  
 Microscopic report as under I, 17.
- VIII 1 Ev. jap.  
 17 January 1907 -- start of experiment.  
 20 January -- water in vessel dries out.  
 21 January -- fresh water refill.  
 22 January -- abundant defoliation: 38 leaves fell off spontaneously, 3 are still holding on but have already burst the epidermis along the absciss zone.  
 Microscopic report: no isolated cells were found (turgescence mechanism).
- VIII 2 Ev. jap in CO<sub>2</sub>-free air; some of the leaves had no leaf blade.  
 17 January 1907 -- start of experiment.  
 21 January -- spontaneous separation of bladeless leaf stems.  
 25 January -- one leaf fell off and 15 remained.  
 28 January -- leaf fall.  
 1 February -- 9 leaves already fallen off, the other seven have gaping crack in absciss zone. In addition to the firmly adhering tube cells, the defoliation wounds are also covered with macerated cells having a round, tube-shape, or very irregular form (I hope to be able to record further details on the cell forms occurring in connection with the various maceration processes in a subsequent study).
- VIII 5 Ev. jap. WS 30°. Air moist. a -- simply suspended; b and c -- standing in water with cutting surface.
- |        | a   | b          | c          |                                    |
|--------|---|------------|------------|------------------------------------|
|        | 17 January 1907   | 17 January | 21 January | Start of experiment                |
|        | 21 January  | 21 January | 25 January | Spontaneous dropping of all leaves |
| VIII 7 | Cinnamomum Reinwardti in CO <sub>2</sub> -poor room; 22 January-1 March 1907. |            |            |                                    |

During more than 5 weeks of experimentation, the concentrated KOH in the crystallization cup was replaced with fresh one only once while the glass bell had to be picked up repeatedly in order to change the water because of threatening mold forma-

tion and in order to freshen up the cutting surfaces. This means that the room should have been approximately CO<sub>2</sub>-free only during a very short period of time. (I think I ought to emphasize this especially because the duration of the experiment was almost as long as under otherwise identical circumstances in the presence of CO<sub>2</sub> (start of defoliation in the 6th week); as far as the maceration of the absciss cells, which otherwise was never observed in *Cinnamomum Reinwardti*, is concerned, we can undoubtedly recognize the consequences of a change brought about by a CO<sub>2</sub> shortage.)

- |      |    |   |
|------|----|---|
| VIII | 8  | <i>Stiftia chrysantha</i> . WS 30°. Air humid; 22-25 January 1907.  |
| VIII | 12 | <i>Eugenia Ugni</i> . WS 30°, Air humid; 23-29 January 1907.  |
| VIII | 16 | <i>Nerium Oleander</i> WS 30°, air humid. 31 January 1907 -- start of experiment. 8 February -- 2 leaves fell off due to contact. 9 February -- remaining leaves fell off spontaneously.  |
| IX   | 1  | <i>Nerium Oleander</i> . WS 30°. Air moist; 13-22 February 1907.  |
| IX   | 2  | <i>Taxus baccata</i> . WS 30° (Air humid until 22 February). 18 February 1907 -- Start of experiment. 22 February -- individual needles fell off upon contact. The room is getting dry. Until 1 March the spontaneous leaf fall was rather minor while many needles could be taken off through contact. |

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#### PLATE CAPTIONS

Figure A. Burst up after drying of youngest internodes (tv), after 2½ weeks in dry room air; the portion tv, which is already separated in the parychema at t broke off completely during preparations for photographing.

Figure B. Same sprout after dropping of tv in absolutely moist room, 10 days later; the uppermost axillary buds (kk') have separated which leads to burst up at a(a' -- cell masses welling up).

Figure C. Same conditions as at A; the burst up developed at b, without dropping portion bs; but the sprout was placed in the humid room only after a swelling had formed at c as a result of the progressing destruction of the axillary buds n; this is why the form of the burst up (c) differs from the one that developed in the dry room (Figure A, t; Figure C, b).

Figure D. Burst up developing in absolutely moist room (lobe formation due to radial cracks in the bark).

Figure E.) Abnormal forms of burst up in dry room air; in Figure F.) Figure F we have a circular swelling right below the cutting surface, in Figure E this is found in the normal position between axillary buds whereby, however, the bark at the same time has ripped open a number of furrows which are rather shallow, broad and which run upward (bark proliferations, Kuster); the sprout shown in Figure E has an abscised layer which penetrates the marrow on the level of the swelling.

Figure G. During decapitation, the upper end of the sprout was cut in half; one half, along with the axillary bud, was removed (d') and the sprout was left to stand in dry room air. (This experiment was intended to give us a new insight into the relationship between the axillary bud loss and the formation of the burst-out; it did not produce any result because

bud d separated earlier, in other words, before a reaction became noticeable in e; but a swelling did form in the vicinity of the wound surface, only on one side at d'.) After organic separation of the second axillary bud (d) the object was placed in an absolutely humid room; a typical burst up developed at e (like the one shown in Figure D); this was followed by a second burst up at f after development of the absciss layers along the buds at e. (The somewhat differing shape here is perhaps the result of its position along the boundary between two annual sprouts.)



Fig. A

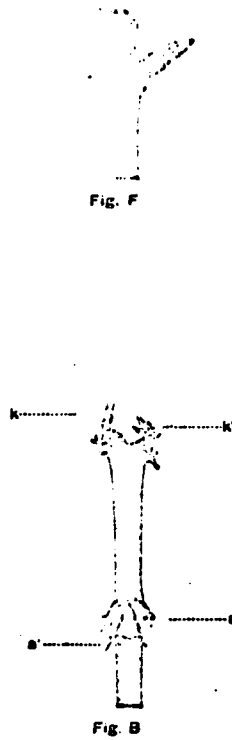


Fig. B



Fig. C

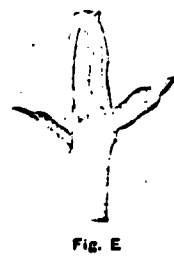


Fig. E



Fig. D

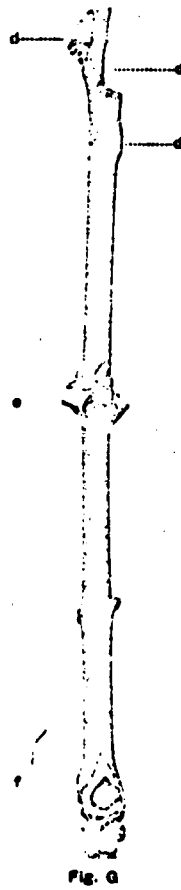


Fig. G